

Original Article

Nitazoxanide stabilizing hypsochromic shift based method for its determination in bulk and in pharmaceutical formulation

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ARTICLE INFO

Received 30 Oct 2014

Revised 20 Nov 2014

Accepted 01 Dec 2014

Keywords:

- Nitazoxanide
- Hypsochromic shift
- Bulk drug
- Tablet
- Validation

ABSTRACT

Nitazoxanide used as antiprotozoal agent. There is no method for determination of the drug content in dosage form without drug was acid stabilized and Hypsochromic shift based method. Simple, fast, reliable and Hypsochromic shift based spectrophotometric method has been developed for determination of nitazoxanide in bulk and tablet dosage forms. The quantitative determination of drug was carried out using the zero order values (absorbance) measured at 343.5 nm in 20% v/v 0.1M Citric acid solutions in Methanol. Use of citric acid in solvent system shift the maximum absorbance wavelength to lower side (Hypsochromic shift) and drug was stable in the solvent system (acid stabilized drug). Drug content was determined in within the desirable confidence interval of 98-102%. The proposed method is economic, sensitive, accurate, reproducible and useful for the determination of nitazoxanide in bulk drug and tablet formulations. The method was validated as per ICH guidelines. The proposed method is economic, accurate, reproducible and useful for the determination of nitazoxanide in bulk drug, tablet formulations, biological fluids, dissolution studies, bio-equivalence studies as well as routine analysis in pharmaceutical industries.

1. INTRODUCTION

Nitazoxanide (NTZ), chemically, 2-acetyl-N-(5-nitro-2-thiazolyl) benzamide (Figure 1), is a synthetic nitrothiazole derivative used as antiprotozoal agent. Nitazoxanide is effective in broad range and protozoal infections. The antiprotozoal activity of nitazoxanide is due to interference with the pyruvate ferredoxin oxidoreductase (PFOR), enzyme dependent electron transfer reaction that is essential to anaerobic energy metabolism. [1,2].

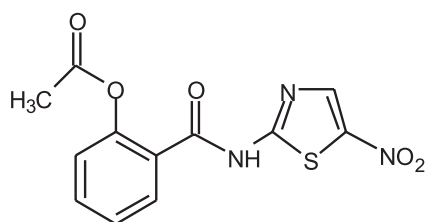


Fig. 1. Structure of Nitazoxanide

Nitazoxanide (NTZ) is a novel broad-spectrum antiparasitic agent originally discovered in 1980s by J.F. Rossignol. NTZ was approved by the US Food and Drug Administration (FDA) in 2002 for use in human beings [3]. It is used for treating both intestinal protozoal infections and helminthiasis [4]. It is also used for treating diarrhea caused by *Giardia lamblia* as well as for cryptosporidiosis in immune-compromised patients, including those with AIDS or HIV infection [5-9].

Most of the study for determination of nitazoxanide based on liquid chromatography (LC) [10-13], LC-mass spectrometry (LC-MS) [8,14], high performance-thin layer chromatography (HPTLC), UPLC [15] and RP-HPLC [16] and spectrophotometric methods (colorimetric method) for estimation of nitazoxanide in pharmaceutical formulation These methods was based on the formation of colored chromogens [17]. These methods were based on the reaction of reduced nitazoxanide with p-dimethylamionobenzaldehyde, p-dimethyl

amino cinnamaldehyde and vanillin in acidic condition to form pink, orange yellow color chromogens [18].

Although various analytical methods have been reported for determination of NTZ in bulk as well as in pharmaceutical formulations, the reported chromatographic methods necessitate sample pretreatment and time-consuming extraction steps prior to analysis of the drug [19] and methods need chemically derivatization to form color chromogens, therefore methods required complex reactions. Chromatographic and Colorimetric methods have been developed for determination of nitazoxanide in present study, several of these methods require the use of hazardous and expensive chemicals, which make the process not only a challenge for the environment but also complex. Moreover, these methods require expensive equipments and considerably skilled personnel. An attempt has been made to develop a simple, reliable, and economical and Hypsochromic shift based spectrophotometric method for determination of nitazoxanide in bulk and pharmaceutical formulations.

2. EXPERIMENTAL

Instrumentation

A Elico UV-VIS Spectrophotometer (Elico SL160) with matching quartz cells 10 mm optical path length, Spectral bandwidth of 1.8 nm and wavelength accuracy of 0.5 nm was used for all absorbance measurements.

Chemicals and reagents

All chemicals and reagents were of analytical reagent grade. Working standard was used as NTZ (Nitazoxanide) was kindly supplied by Alembic Pharmaceutical, Vadodara for providing the gift samples of the drug and was used as the reference standard.

Preparation of standard solution

10 mg of nitazoxanide was accurately weighed, transferred to 100 ml volumetric flask, dissolved in reference solvent and volume was made-up to 100 ml with reference solvent to obtain stock solution of drug of concentration of 100 µg/ml. Working standard solutions of nitazoxanide were prepared by diluting different volumes of stock solution (100 µg/ml) in a 10 ml volumetric flask to give a concentration range of 2.5 to 20 µg/ml using reference solvent.

Preparation of sample solution

Twenty of label claimed 500 mg of NTZ of same batch were triturated in a mortar until fine powder. Accurately weighed amount of powder equivalent to 20 mg of nitazoxanide was transferred in 100 ml volumetric flask containing small quantity of reference solvent. Ultrasonic water bath was used for 20 minutes to complete dissolution. The solutions were diluted to volume and filtered through Whatman filter paper no.40. Further suitable dilutions were made to obtain six replicates of 10 µg/ml solutions. These solutions were analyzed and amount of nitazoxanide was determined.

Method validation

According to ICH guidelines [20,21] the developed analytical method has been validated for specificity, linearity, precision, limit of detection, limit of quantification, accuracy and as mentioned below.

For specificity study, any possible bias was determined by comparison of result of assay value obtained in presence of excipients to assay value without excipients. Assay bias was evaluated by calculating the percentage agreement.

The linearity was determined by serial dilutions (2.5-20 µg/ml) of the NTZ in reference solvent (methanol: 0.1 M citric acid 80:20). The determination was repeated three times at each concentration level. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision of the method was studied under the head of repeatability, intraday and interday precision. In repeatability, the absorbance of solution (5 µg/ml) was measured six times and recorded. Solutions were containing 5 µg/ml, 10 µg/ml and 20 µg/ml of nitazoxanide. The absorbances of these solutions individually were measured at thrice within a day and three consecutive days for intraday and interday respectively.

The accuracy of the method was assessed, based on recovery studies. The technique of standard addition was used to assess accuracy of the method. For the purpose a concentration of 7.5 µg/ml was selected to prepare the sample matrix of the bulk drug and marketed dosage form respectively.

Analysis of dosage form

The estimation of the pharmaceutical dosage form was determined by the proposed validated method. Accurately weighed 51.12 mg powder (equivalent to 20 mg of NTZ) was transferred in 100 ml volumetric flask containing small quantity of reference solvent. Ultrasonic water bath was used for 20 minutes to complete dissolution. The solutions were diluted to volume and filtered through Whatman filter paper no.40. Percent drug content in tablets were determined by extrapolating the absorbance values in regression line.

3. RESULTS AND DISCUSSION

A simple, rapid, acid stabilized and Hypsochromic shift based UV spectrophotometric assay method has been developed and validated for analysis of nitazoxanide (NTZ) in pharmaceutical formulation.

Optimization, solution stability and selection of solvent system

After allowed to stand, visually examined and scanned of nitazoxanide in various solvent systems were done, a clear transparent solvent was obtained and stable spectra of nitazoxanide in methanol: 0.1 M citric acid (80:20) for 240 minutes or above was shown, stability of nitazoxanide in this solvent system.

Determination of wavelength of maximum absorbance (λ_{\max}) of Nitazoxanide

The absorption spectra of standard solution of nitazoxanide in the range of 2.5-20 $\mu\text{g/ml}$ were scanned in the wavelength range of 220-430 nm against reference solvent as blank (Table 1).

The Overlain spectra of 2.5 - 20 $\mu\text{g/ml}$ of standard nitazoxanide solutions in reference solvent are, shown in (Figure 2). The determination of wavelength of maximum absorption was better selected from overlain spectra of different concentration of drug solution. Overlain Absorption Spectra of nitazoxanide absorption wavelength 343.5 nm was selected as λ_{\max} for assay.

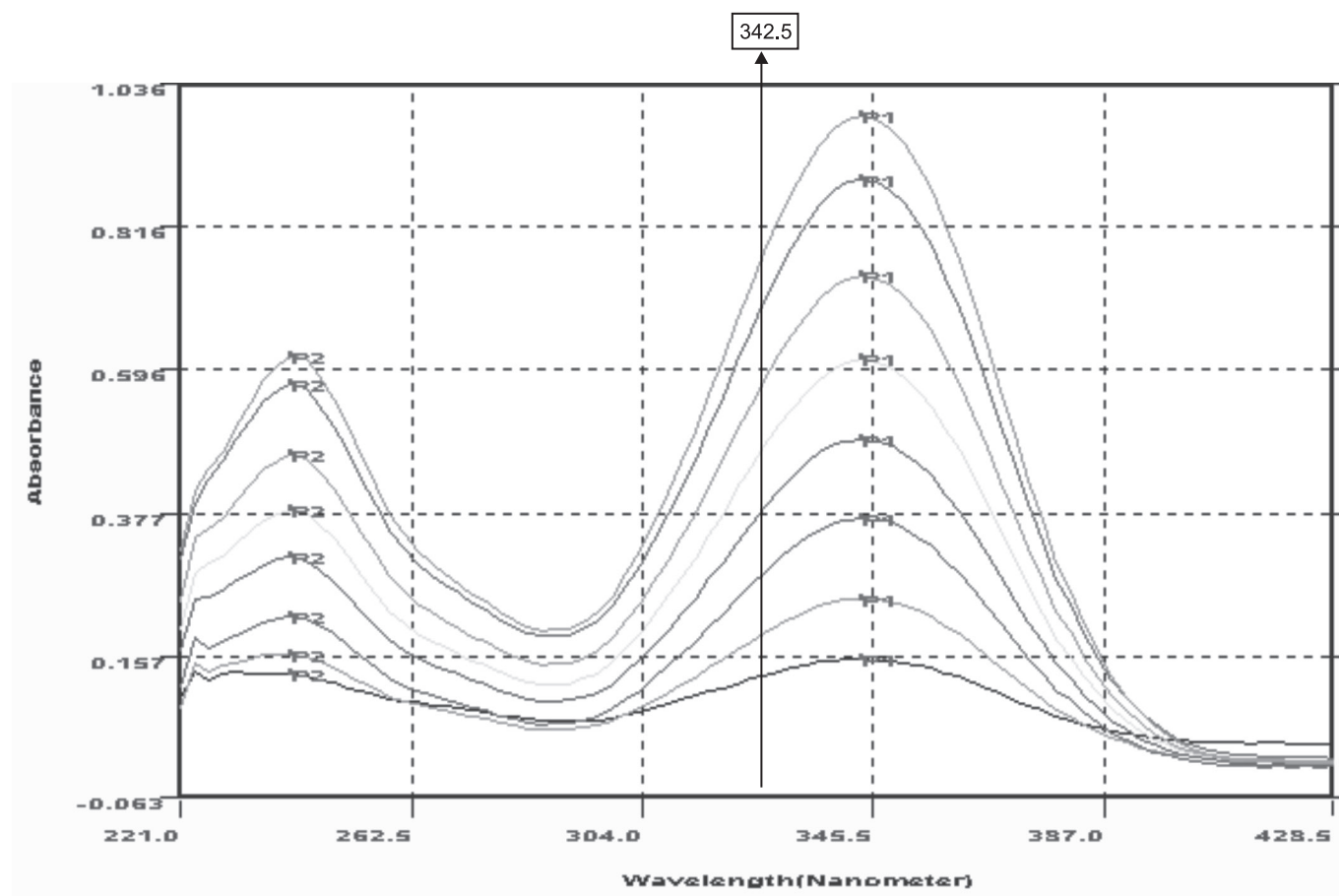


Fig. 2. Overlain spectra of 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 $\mu\text{g/ml}$ of standard Nitazoxanide solutions in reference solvent.

Table 1. Calibration curve data for Nitazoxanide

S. No.	Conc. ($\mu\text{g/ml}$)	Abs.*
1	2.5	0.119
2	5	0.249
3	7.5	0.384
4	10	0.507
5	12.5	0.628
6	15	0.764
7	17.5	0.899
8	20	1.015

*Data represents mean of triplicate determinations.

Method validation

Specificity

In order to verify the absence of interference of excipients on the analysis of NTZ tablets a sample was prepared with all the excipients present in the tablets but without the drug (placebo). Absorption spectra did not show any potential interference of the tablet excipient at λ_{\max} of 343.5 nm.

Linearity and range

Regression analysis using the method of least square was made for slope, intercept and correlation coefficient value. Calibration data is analysed statistically involving application of various statistical tests like test for linearity, test of slope, test of intercept, test of correlation coefficient and their confidence intervals are determined. (Table 2).

Table 2. Statistical tests result of linearity studies

S.No.	Test parameter	Formula	Result and discussion
1.	Test of correlation coefficient	$t = r/\sqrt{(1-r^2)/N-2}$ $t = [0.9999 * (24-2)^{1/2}] / (1-0.9998)^{1/2}$ t calculated (331.6) value > table value (2.074) (at 5% level for d.f. 22)	There is significant correlation between absorbance and concentration. Hence it passes the test of correlation coefficient.
2.	Test of intercept	$t = \text{intercept} - 0 / [S^2 (1/N + x^2/\sum(X-x)^2)]^{1/2}$ $t = 0.007 - 0 / [0.000809(1/24 + 0.4821)]^{1/2}$ t calculated (0.341) value < table value (2.074) (at 5% level for d.f. 22)	Hence it is concluded that intercept is not significantly different from zero.
3.	Test of slope	$t = b - 0 / [S^2/\sum(X - \bar{X})^2]^{1/2}$ $t = 0.0513 - 0 / [0.000809/262.5]^{1/2}$ t calculated (29.65) value > table value (2.074) (at 5% level for d.f. 22)	It is concluded that the observed slope is significantly different from zero.

A Calibration curve (Figure 3) was constructed at optimum experimental conditions using absorbance versus concentration in the range 2.5-20 µg/ml. High value of correlation coefficient (0.9999) indicates good linearity and adherence of method to Beer's law. Beer's law data and regression characteristics are determined. (Table 3).

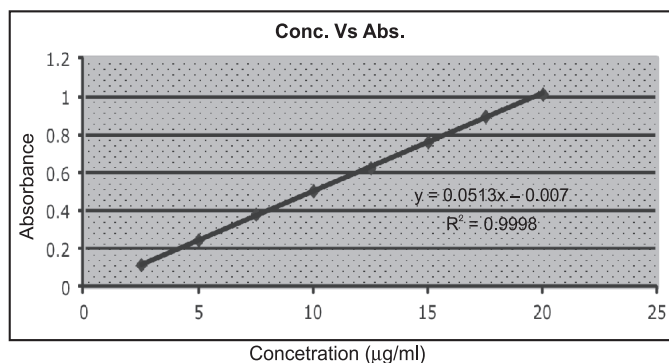


Fig. 3. Calibration curve of Nitazoxanide at 343.5 nm.

Table 3. Beer's law data and regression characteristics

Parameters	UV spectrophotometric methods
Regression equation ^a	$Y = 0.0513x - 0.007$
Slope (b)	51.3×10^{-3}
Intercept (a)	7×10^{-3}
Linearity range (µg/ml)	2.5–20
Molar absorptivity (Lit/mole/cm)	15.764×10^3
Correlation Coefficient (r)	0.9999

^a means $y = a + bC$, where 'C' is the concentration in (µg/ml) and y is absorbance unit.

Precision

The method shows good repeatability that is demonstrated by RSD of lower than 0.62%. The RSD was found to be within acceptance limit (Table 2).

For Intraday and interday, precision of the method, solutions of nitazoxanide were prepared at three concentration levels 5, 10, 20 (µg/ml) each in triplicate. The mean RSD (%) for Intraday 1, 2, and 3 were found to be 0.34, 0.44, and 0.33, respectively and for Interday 1, 2, and 3 were found to be 0.33, 0.61, and 0.53, respectively. The RSD was found to be within acceptance limit (<2%), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, method at selected wavelength was found to be precise (Table 2).

Accuracy

The accuracy of the method was assessed, based on recovery studies. An accuracy criterion for an assay method is that the mean recovery should desirably be $100 \pm 2\%$ at each concentration over the range of 80-120% of target concentration. Since the mean % recovery varies from 99.0 to 99.8% and are within the desirable confidence interval of 98-102%, it can be said that the proposed method is accurate (Table 2).

Limits of detection (LOD) and limit of quantification (LOQ)

The developed method was highly sensitive to detect and determine the NTZ content. The LOD for S/N ratio of 3:1 was 0.12 µg/ml and the LOQ for signal to noise ratio of 10:1 was 0.39 µg/ml (Table 2).

Analysis of dosage form

After the validation of the newly developed UV spectrophotometric method, the estimation of marketed formulations was determined by the proposed validated method and the results are shown in Table 4. Among the different marketed brands used, the estimation of the brands was found to be 98.23 and 98.50% of labeled amount (Table 5). According to USP 2011, within specified limits of 98-102%, it can be said that the proposed method can satisfactorily be applied for routine analysis of nitazoxanide in tablet dosage form.

Table 4. Validation parameters of developed method for NTZ

Parameters	Values
Linearity(µg/ml)	2.5-20 µg/ml
Correlation coefficient	r ² = 0.9998
Accuracy (% Recovery)	99.0-99.8
Precision	
Repeatability (% RSD)	<0.62
Intraday (% RSD)	<0.44
Inter-day (% RSD)	<0.61
LOQ (µg/ml)	0.12
LOD (µg/ml)	0.39
Specificity (Average agreement %)	101.10

Table 5. Result of assay of NTZ dosage form

Brand	Labeled amount (mg)	Amount found a (mg)	% of Labeled amount ^a	% RSD
Nizonide - 500	500	493.5± 1.210	98.23 ± 0.796 (98–102 %)	0.245

^adata represents mean ± SD; n = 6

4. CONCLUSION

Hence, the new proposed stabilizing Hypsochromic shift based method was developed and validated and results obtained and the statistical parameters for determination of NTZ in pharmaceutical dosage forms demonstrated that method is simple, accurate, fast and precise. The method showed high sensitivity, acceptable linearity and accuracy. Therefore it could be easily used for the analysis of pure drug. Moreover, the method uses simple reagents with minimum steps and time for sample preparation, which allow it to be useful for routine analysis and quality control assays of NTZ in tablet dosage form.

Competing interests

The authors declare that they have no competing interests.

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